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6-Cyclopropylpurines as Novel Potent Analogs of Cytokinins

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Abstract

6-Alkynyl-, *trans*-6-alkenyl-, *trans*-6-cyclopropyl- and 6-alkylpurines structurally related to the cytokinin 6-benzylaminopurine (BAP) have been synthesized and examined with a radish cotyledon assay as plant growth stimulators. The growth stimulation obtained with the 6-alkylpurines *trans*-cyclopropylpurines was very close to that obtained with BAP, and the *trans*-styrylpurines were somewhat less effective. The fact that the conformationally locked cyclopropanes exhibit growth-stimulating effects comparable to the flexible 6-alkylpurines and to BAP, supports the hypothesis that the orientation of the NH-CH_2 bond in ''the active conformation'' of BAP is close to *anti*, which means that the torsion angle C(6)-N(6)-CH_2-C is approximately 180 degrees.

Key words: Cytokinin; Plant growth; Radish cotyledon; 6-Cyclopropylpurine; Conformation; Synthetic analogs

Introduction

Cytokinins (CK) are plant growth hormones that promote cell division and cell growth. 6-Benzylaminopurine (BAP) and *trans*-zeatin (Figure 1) are among the most potent known naturally occurring CKs. Metabolism of zeatin involves cleavage of the side chain by the enzyme system cytokinin oxidase/dehydrogenase (CKX) (Galuszka and others 2001); adenine, without any phytohormone properties, is formed irreversibly. BAP is also metabolized to adenine, but knowledge about the enzyme system is limited. 6-Substituted purines lacking the exocyclic amino functionality in the 6-position are not expected to be substrates for CKX and similar enzymes. A prolonged cytokinin effect is therefore expected.

Synthetic BAP analogs where the NH-CH_2– part of the side chain is replaced by a C ≡ C, *trans* CH=CH or CH_2–CH_2 fragment have been examined as potential plant growth stimulators. Compound **1b** with the saturated and hence flexible side chain was found to act as a cytokinin. Depending on the assay used, the activity is comparable (Henderson and others 1975) or somewhat lower than that of BAP (Nishikawa and others 1986). Also, the *trans*-styrylpurine **2b** is highly active, in the lettuce germination assay, and in the *Amaranthus* betacyanin test, activity comparable to BAP was found (Koyama and others 1985). Another group using the tobacco callus assay found compound **2b** to be somewhat less potent than **1b** and BAP (Henderson and others 1975). The *trans* styrylpurine **2b** is significantly more potent than the *cis*-isomer (Koyama and

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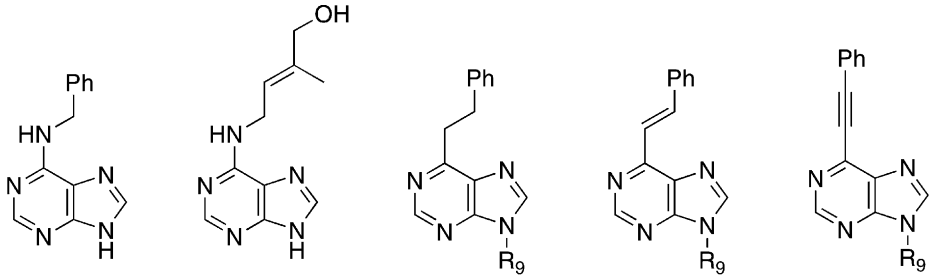


Figure 1. Structure of naturally occuríng cytokinins and synthetic analogs.

others 1985). The alkynylpurine **3b** was reported to be essentially without growth-promoting activity (Koyama and others 1982; Nishikawa and others 1985). These results demonstrate that the NH-functionality in the purine 6-position is not necessary for cytokinin activity. Furthermore, the results may indicate that the orientation of the NH-CH_2 bond in ''the active conformation'' of BAP is close to *anti* which means that the torsion angle C(6)-N(6)-CH_2-C is approximately 180 degrees. It has also been suggested that the biologically active conformations of CKs should adopt the conformations found by X-ray. In the crystalline CKs the torsion angles C(6)-N(6)-CH_2-C are 80–100 degree (Bugg and Thewalt 1972; Korszun and others 1989; Raghunathan and others 1983; Soriano-García and others 1987; Soriano-García and Parthasarathy 1977). Further structure–activity studies of 6-substituted purines with side chains of various flexibility may reveal important information regarding receptors and which conformations are ''active'' in the naturally occurring cytokinins. The cyclopropyl ring may be regarded as a bioisoster (functional groups or molecules that have chemical and physical similarities producing broadly similar biological properties) for the carbon-carbon double bond. We here report the first synthesis of 6-phenylcyclopropylpurines and their cytokinin activity compared to BAP and the BAP analogs **1–3**.

Materials and Methods

Silica gel for flash chromatography was available from Merck (Darmstadt, Germany) (Merck No. 9385) or Fluka (Fluka No. 60752). Tetrahydrofuran (THF) was distilled from Na/benzophenone. The ^1H nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz with a Bruker Avance DRX 500 instrument, or at 200 MHz with a Bruker Avance DPX 200 instrument. The ^{13}C NMR spectra were recorded at 125 MHz or 50 MHz with the same instruments. Unless otherwise stated, the spectra were recorded at ambient temperature. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane. Mass spectra were recorded with a VG Prospec instrument at 70 eV ionizing voltage, and are presented as *m/z* (% rel. int.). Melting points are uncorrected. Compounds available by literature methods: **1a, 1b, 3a**, and **3b** (Bråthe and others 2002), **2a** and **2b** (Bråthe and others 1999), and **4** (Robins and others 1961).

6-[(*E*)-2-Phenylcycloprop-1-yl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (5a).

(*E*)-1-Bromo-2-phenylcyclopropane (Lang and Brandsma 1998) (473 mg, 2.4 mmol) was dissolved in dry THF (1.5 ml) and cooled to −89°C. *n*-BuLi (1.5 ml, 1.6 M in hexane) was added, and the reaction mixture was stirred for 50 min before a solution of $ZnBr_2$ in THF (2.4 ml, 2.4 mmol, 1.0 M) was added. After an additional 50 min, the mixture was allowed to reach ambient temperature. In another flask, 6-iodo-9- (tetrahydro-2*H*-pyran-2-yl)-purine **4** (660 mg, 2.0 mmol), $(dba)_3Pd_2CHCl_3$ (52 mg, 0.0502 mmol), and triphenylphosphine (105 mg, 0.40 mmol) was dissolved in dry THF (3 ml) and stirred until a transparent yellow solution was obtained. The purine solution was transferred to the flask containing the zinc reagent, and the resulting mixture was refluxed for 3 h and poured into sat. aq. NH_4C1 (35 ml). The mixture was extracted with chloroform (3 × 30 ml), and the combined organic phases were dried ($CaCl_2$) and evaporated. The crude product was purified by flash chromatography on silica gel. EtOAc (0–65%) in hexane was used as eluent; yield 440 mg (69 %) colorless crystalline solid, mp 118°–120°C. ^1H NMR (200 MHz, $CDC1_3$, 20°C): δ 8.86 (s, 1H, H-2), 8.25 (s, 1H, H-8), 7.37–7.17 (m, 5H, Ph), 5.81 (m, 1H, THP), 4.22 (m, 1H, THP), 3.83 (m, 1H, THP), 3.11 (m, 1H, cyclopropyl), 2.95 (m, 1H, cyclopropyl), 2.15 (m, 3H), 1.75 (m, 5H); ^{13}C NMR (50 MHz, $CDC1_3$, 20°C): δ 162.3, 152.5, 149.38, 149.36, 141.25, 141.22, 141.20, 132.3, 128.3, 126.1, 126.08, 126.05, 81.9,

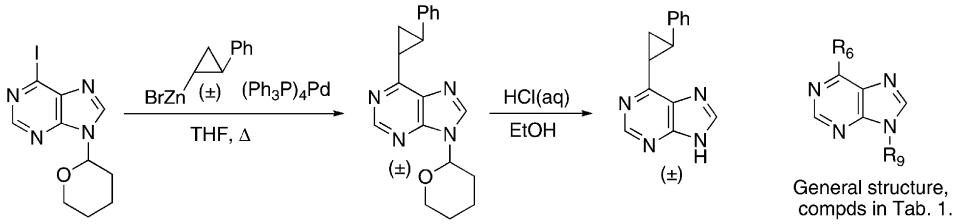


Figure 2. Synthesis of the 6-cyclopropylpurines **5**.

81.8, 68.78, 68.77, 31.8, 31.76, 29.54, 29.48, 24.8, 24.7, 22.7, 20.3, 20.2; MS (El) *m/z* (rel. %): 320 (M^+, 2), 237 (19), 236 (100), 235 (55), 221 (6), 208 (6), 159 (13), 134 (30), 115 (10), 91 (7); HRMS: Found 320.1630, calcd. for $C_{19}H_{20}N_4O$ 320.1637; Anal: Found: C, 70.94; H, 6.51; N, 17.10. $C_{17}H_{18}N_4O$ requires C, 71.23; H, 6.29; N, 17.49%.

6-[(*E*)-2-Phenylcycloprop-1-yl]-1*H*-purine (5b)

6-(*E*)-2-Phenylcycloprop-l-yl)-9-(tetrahydro-2*H*-pyran-2-yl)-purine **5a** (282 mg, 0.88 mmol) in EtOH (20 ml) and HC1 (15 ml, 1M) was stirred at ambient temperature for 2 h, neutralized with solid Na_2HCO_3, and evaporated under reduced pressure together with a small amount of silica gel. The residue was added on top of a flash chromatography column, and the product was eluted with 0–5% EtOH in EtOAc; yield 170 mg (80%) colorless crystalline solid, mp 226°–228°C. ^{1}H NMR (500 MHz, CD_3OD, 50°C): δ 8.74 (s, 1H, H-2), 8.40 (br s, 1H, H-8), 7.27 (m, 2H, Ph), 7.21 (m, 2H, Ph), 7.17 (m, 1H, Ph), 2.93 (br s, 1H, cyclopropyl), 2.82 (m, 1H, cyclopropyl), 2.04 (m, 1H, cyclopropyl), 1.73 (m, 1H, cyclopropyl); ^{13}C NMR (125 MHz, CD_3OD, 50°C): δ 153.6, 145.2 (br), 142.4, 129.5, 127.3, 127.2, 30.4, 25.6, 19.9; MS (EI) *mlz* (rel. %): 237 ($M^+ + 1$, 14), 236 (M^+, 100), 235 (89), 234 (6), 221 (14), 208 (10), 159 (25), 158 (5), 134 (48), 115 (21); HRMS: Found 236.1051, calcd. for $C_{14}H_{12}N$ 236.1062.

Procedure for Determination of Cytokinin Activity

The cytokinin activity of the compounds was determined using a bioassay method described by Letham (1971). Radish (*Raphanus sativus* L. cv. Cherry belle) cotyledons were used as the cytokinin-sensitive plant material. To determine the CK effect of the purines, the weight gains of cotyledons treated with the 100 μM cytokinin analogs were subtracted from the weight gains of control cotyledons (0 μM purine). This effect on growth of radish cotyledons was compared to the effect of BAP (100 μM) and is expressed as percentage of the BAP effect. The results are based on the mean of three replicate dishes of each treatment.

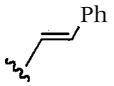
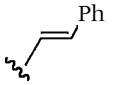
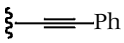
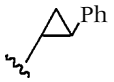
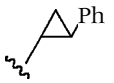
Results and Discussion

To the best of our knowledge, there is only one prior report on the preparation of a 6-cyclopropylpurine, and the reported synthetic strategy was not applicable for our cyclopropane targets **5** shown in Figure 2 (Wanner and others 1978). We applied a palladium catalyzed coupling strategy. *trans*-l-Bromo-2-phenylcyclopropane (Lang and Brandsma 1998) was converted to the corresponding zinc reagent and reacted with the 6-iodopurine **4**. The THP-protecting group in compound **5a** was removed under acidic conditions (Figure 2).

The cytokinin activities of compounds **1**-**3**, **5** as well as BAP were determined using the radish cotyledon assay (Letham 1971). The results are presented in Table 1.

The results confirmed that the alkynes **3** exhibit essentially no growth-stimulating effects in the concentration range studied. At some concentrations, slight growth inhibition could be observed. We have also previously found high cytotoxicity toward certain mammalian cancer cell lines for 6-alkynylpurines (Bråthe and others 2003). The growth stimulation obtained with the 6-alkylpurines **1** was very close to what was obtained with BAP, and the *trans*-styrylpurines **2** were somewhat less effective. The cyclopropanes, especially the THP-protected purine **5a**, were highly active in the radish cotyledon assay. At 10 μM compound **5a** was even slightly more potent than the naturally occurring hormone BAP. The fact that the conformationally locked cyclopropanes **5** exhibit growth-stimulating effects comparable to the flexible 6-alkylpurines **1** supports the hypothesis that the orientation of the NH-CH_2 bond in ''the active conformation'' of BAP is close to *anti*. The lower activity found for the *trans*-styrylpurines **2**, may be attributed to electronic factors rather than shape. For instance, it is well documented that the side chain double bond in 6-alkenylpurines is highly

Table 1. Cytokinin Activity of BAP and Synthetic Purines 1–5

Compound no.	R_6	R_9	% Weight increase relative to BAP at 1 μM conc[a]	% Weight increase relative to BAP at 10 μM conc[a]	% Weight increase relative to BAP at 100 μM conc[a]
BAP	$-NHCH_2Ph$[b]	−H	100	100	100
1a	$-CH_2CH_2Ph$	−THP[c]	52	67	89
1b	$-CH_2CH_2Ph$	−H	58	67	92
2a		−THP	24	−2	36
2b		−H	41	35	64
3a		−THP	−9	−10	−4
3b		−H	−17	−15	1
5a		−THP	62	110	90
5b		−H	84	76	71

[a] *Comparison of weight gain between radish cotyledon grown without any purine added or at 1, 10, or 100 μM purine concentration*
[b] *Ph = phenyl*
[c] *THP = tetrahydropyran-2-yl.*

electron deficient and prone to nucleophilic attack (Øverås and others 1997). In this communication it is reported, for the first time, that 6-cyclopropylpurines are highly active plant growth stimulators.

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